Cancer Chemother Pharmacol (1990) 27: 121–124

Cancer Chemotherapy and Pharmacology

A new intraperitoneal tumor model in the rat

J. D. Nagel[1, 2], G. Los[1], A. C. Begg[1], and J. G. McVie[1, 3]

[1] Division of Experimental Therapy, The Netherlands Cancer Institute, Antoni van Leeuwenhoekhuis, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
[2] Present address: Department of Haematology, Oncology and Clinical Immunology, Medical Faculty, University of Düsseldorf, Federal Republic Germany
[3] Present address: Cancer Research Campaign, 2 Carlton House Terrace, London SW1Y 5AR, U.K.

Received 28 August 1989/Accepted 13 August 1990

Summary. A new tumour model that is particularly suitable for testing intraperitoneal chemotherapy is described. Single tumours were induced to grow in the mesentery of rats by the implantation of small pieces taken from subcutaneous tumours. Tumour growth was monitored by repeated laparotomies at which the tumour size was measured with calipers. In this way, growth curves of treated and untreated tumours could be defined. The diameter of untreated intraperitoneal tumours increased linearly with time [diameter (mm) = 0.39 t (days) +2.4]. Tests using different numbers of laparotomies showed that the procedure itself had little influence on growth. Cell kinetic studies of 6-mm tumours showed a mean labelling index of 31% and a volume-doubling time of 3.9 days, resulting in cell-loss factors probably in excess of 70%. The model was tested by assessing the effect of the chemotherapeutic agent cisplatin. Regression and regrowth could be satisfactorily followed, leading to estimates of growth delay. This model therefore provides a quantitative way to assess the response of intraperitoneal tumours to chemotherapy.

Introduction

Intraperitoneal chemotherapy for cancers restricted to the peritoneal cavity has demonstrated pharmacokinetic advantages over intravenous therapy, both in experimental animals and in the clinic [3]. There is a need, however, for suitable animal models that measure the effectiveness of different forms of therapy. One relevant and quantitative parameter for measuring therapeutic effectiveness is the delay in tumor growth [1, 10]. Commonly used rat models are those of intraperitoneal metastases after intraperitoneal injection of a tumor-cell suspension [5, 6]. Such models are not suitable for measuring the delay in tumor growth, however, because they provide multiple metastases of various sizes and at different, often unmeasurable, sites. The new model described provides a single intraperitoneal tumor that is located at a defined site in the mesentery. The antitumor effect of different forms of treatment can be readily compared by measuring the growth delay of the tumor that is assessable by repeat laparotomy. One index treatment-induced toxicity, as with other tumor models, can be measured concurrently by monitoring body-weight changes.

This paper reports on the methods for inducing and monitoring the tumours, their cell kinetic properties and their reponses to an intraperitoneally injected drug.

Materials and methods

Experimental animals and husbandry. SPF Wag/Rij rats aged 3 months and weighing 270–290 g were used. Animals were acclimatized for at least 3 weeks in rooms with controlled conditions (artificial lighting from 0700 to 1900 hours; ventilation: 15 air changes/h, 22°C and relative humidity of 55%). Rats were housed in polycarbonate cages (Makrolon type III, three rats per cage) on presterilized wood shavings. They were given Hope Farm AM II food (Woerden, The Netherlands) and plain tap water ad libitum.

Tumor model. The CC531 tumor, a moderately differentiated adenocarcinoma of the rat colon, was used throughout the study. The tumour was originally induced by six weekly injections of 30 mg/kg 1,2-dimethylhydrazine (DMH) into a Wag/Rij rat. It arose in the proximal colon 40 weeks later [7] and was kept in culture flasks in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS). A single-cell suspension was prepared by trypsinization, counting in a haemocytometer, centrifuging (1,000 rpm, 5 min) and suspending in phosphate-buffered saline (2×10^6 cells/ml). All cell suspensions contained over 95% viable cells as assessed by trypan blue exclusion.

Preparation of tumor pieces for implant. A tumor-cell suspension (1 ml) containing 2×10^6 cells was injected into the flank of the rats. After 3 weeks a solid tumor with a diameter of approximately 3 cm was produced. The tumor was excised and cut into slices of 1.5 mm. For this purpose, a custom-made tool consisting of five razor blades separated by a constant distance of 1.5 mm was used. The tumor slices showed two different regions: a necrotic centre and a solid, viable outer border. From the grossly viable part, cylinders of 3-mm diameter were punched. Each

Offprint requests to: G. Los

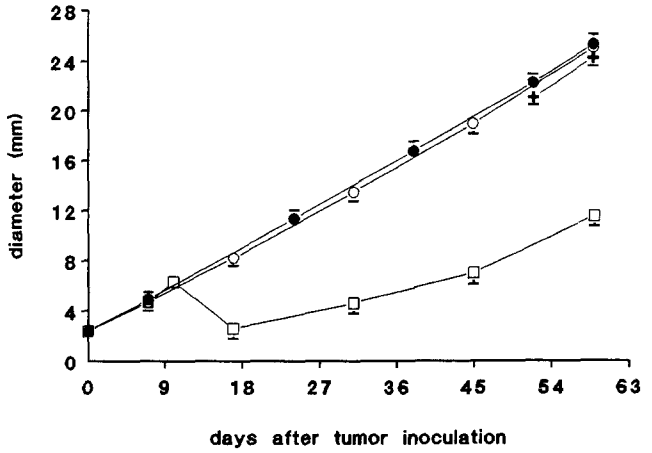

Fig. 1. Growth rates of CC531 tumours grown intraperitoneally in rats. Groups A (●), B (○) and C (+): untreated (see Materials and methods); group D (□): 4 mg/kg cisplatin on day 10

tumour piece therefore measured 3 mm in diameter by 1.5 mm in thickness. The tumor pieces were put in physiological saline and were kept for a maximum of 2 h at room temperature before implantation. For the subcutaneous tumours used for chemosensitivity testing, cells were injected as described above and the rats were treated when the tumours had reached a mean diameter of between 6 and 10 mm.

Intraperitoneal implantation. Rats were anaesthetized with ether. After shaving and preparation of the operation site with 70% ethanol, a 1-cm incision was made into the median ventral abdominal wall, close to the transitions of the upper and lower quadrants. Part of the small intestine was exposed and the tumour pieces were implanted by a haemoclip fixed to the distal part of the mesentery. Care was taken not to occlude the respective mesenteric vein. The bowel was repositioned and the abdomen, closed by 3–4 simple interrupted sutures.

Intraperitoneal tumour characterisation. Solid intraperitoneal tumours were not necrotic during the period of observation (63 days). However, large (approximately 2 cm) tumours began to develop small necrotic centres. This tendency was significantly lower in comparison with subcutaneously growing tumours. Tumour pieces were implanted in highly vascularized locations, resulting in ingrowing of blood vessels from the mesentery into the tumour. As the tumours grew, they were covered by connective tissue containing a tight network of blood vessels. This relatively rich vascular supply is probably the reason why necrotic centres occurred only in large intraperitoneal tumours. Metastases were not detected during the observation period of 63 days.

Tumour measurements. A laparotomy was performed once every 2 weeks to measure the intraperitoneally growing tumour. The rats were anaesthetized with ether and, after the small intestine had been exposed, tumour size was assessed by measuring the three perpendicular diameters of the tumour with digital calipers. The geometric mean of the three values was then calculated [1]. The bowel was repositioned and the abdomen, closed as described above. Depending on the experimental group, each rat received several laparotomies. It was therefore necessary to guard against possible infections. Sterilization of the surgical instruments was routine, and Betaisodona solution was used for disinfecting the skin. In addition, every unnecessary irritation of the bowel was avoided, since a mechanical ileus could have been caused. For subcutaneous tumours, three perpendicular diameters were measured using calipers without the need for an operation or anaesthetic.

Cell kinetics. The cell kinetic parameters of intraperitoneal CC531 tumours were measured using the thymidine analogue 5′-iododeoxyuridine (IUdR) and flow cytometry. Rats were injected intraperitoneally with 30 mg/kg IUdR and killed at intervals up to 7 h, at which time the tumours were excised. The methods of preparation and analysis have been described elsewhere [2]. Briefly, tumour pieces fixed in 70% ethanol were treated with pepsin to produce suspensions of nuclei. DNA was partially denatured with 2 *N* HCl and the nuclei were stained with an IUdR-DNA-specific mouse monoclonal antibody (Partec AG, Switzerland) followed by a fluorescein isothiocyanate (FITC)-conjugated anti-mouse antibody. Nuclei were subsequently counter-stained with propidium iodide for total DNA content and then analyzed flow cytometrically for red and green fluorescence simultaneously. Windows placed around selected subpopulations were used to calculate the labelling index (percentage of green fluorescent cells) and the position of the labelled (green) cells relative to G1 and G2 so as to estimate the length of the DNA synthesis phase [2]. Flow cytometry was carried out using a FACScan (Becton Dickinson; Sunnyvale, Calif.).

Treatment. Tumour-bearing rats ($n = 6$) were injected intraperitoneally with 4 mg/kg cisplatin (Bristol Myers; Weesp, The Netherlands) on day 10 after tumour implantation. This dose is 80% of the MTD and was used to avoid possible problems with altered susceptibility of the rats to the drug by the repeated laparotomies. The first laparotomy after treatment was performed on day 17 and the last, on day 59. Six rats were used in the operated controls group and four, in the non-operated control group. For comparison, rats ($n = 3$) with subcutaneous tumours taken from another experimental series were injected i.v. with 5 mg/kg cisplatin; five rats were used as controls.

Results

Four of the initial attempts to implant tumour pieces in the mesentery proved to be technical failures, but after we had familiarized ourselves with the technique, all operated rats could be used for the experiments. The rats used for tumour-growth tests were divided into four groups. Animals in groups A, B and C were not treated, whereas those in group D received 4 mg/kg cisplatin intraperitoneally on the 10th day after implantation. Groups A and B were used to determine growth in untreated rats. As we did not know whether the animals could survive a laparotomy every week, we decided to perform the operation every 2 weeks. To ensure sufficient measurements, the laparotomies were done on alternate weeks for groups A and B, respectively (see Fig. 1). After the implant operation, tumours were followed up over a period of 59 days. The mesenteric tumours in groups A, B and D were measured every other week, whereas the tumours in group C were measured only on days 52 and 59 so as to test the influence of the number of operations on tumour growth.

The sizes of the tumours in groups A and B showed a linear increase in diameter from 2.4 mm at implant to approximately 25 mm on day 59 (Fig. 1). This relationship of tumour diameter with time could be described by the equation:

$$y = 0.39\,t + 2.4,$$

where y represents the size of the mesenteric tumour in millimeters and, t represents the number of days. There was no difference in tumour-growth rate between groups A and B. The tumours in group C were slightly smaller when measured on days 52 and 59, although the differences were small and the growth rate appeared to be identical. This indicated that the number of operations performed in groups A and B had little influence on tumour growth. The cisplatin-treated group showed a marked delay in growth

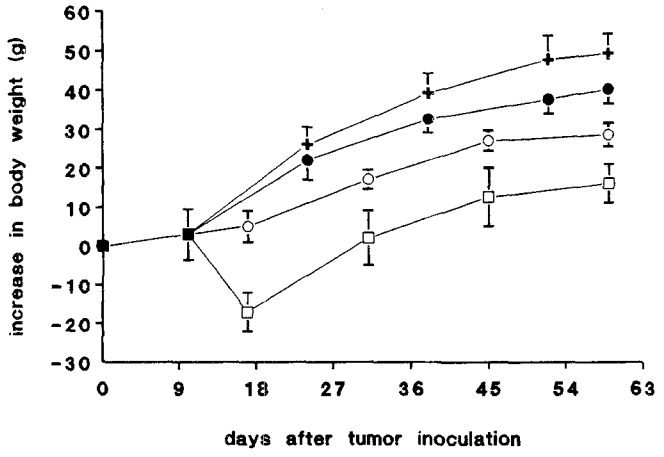

Fig. 2. Change in rat body weight (g). Symbols as in Fig. 1

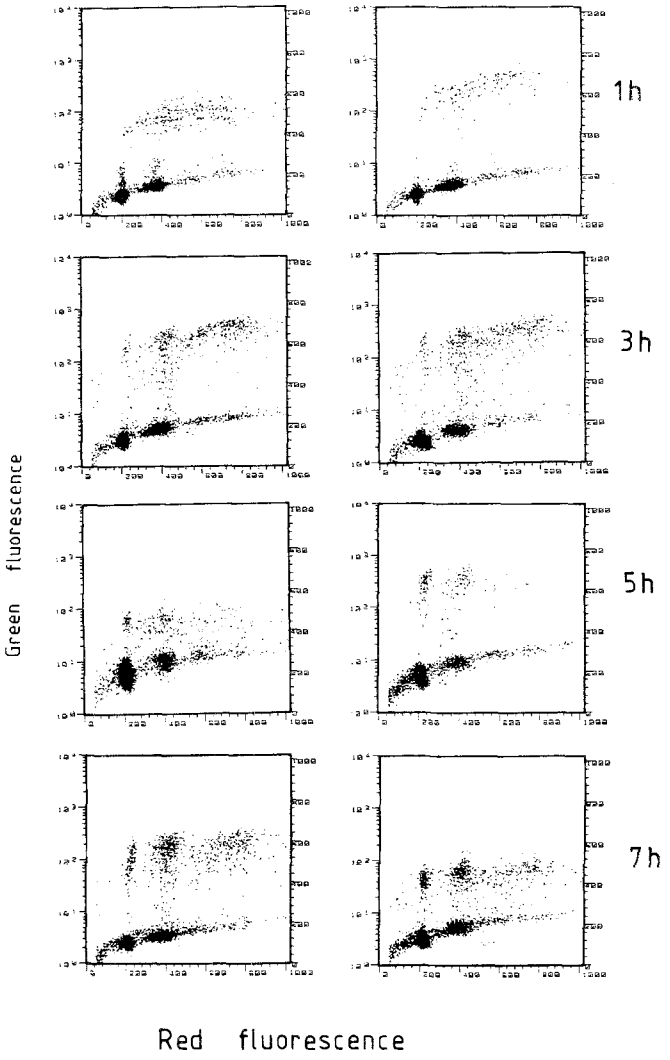

Fig. 3. Flow cytometric cytograms of green (IUdR content) versus red fluorescence (total DNA content) for CC531 tumours. Rats were injected with IUdR and killed at the times indicated

of the tumours. After an initial decrease in tumour size, regrowth occurred after day 17.

Complications from the operation included infections of both the skin and the peritoneum, with the latter leading to peritonitis. Both could be avoided by more attention to sterile technique. Mechanical ileus also occasionally resulted from bowel manipulation and tumour growth in later stages. The former could largely be avoided by careful intervention procedures at each laparotomy. Indications of toxicity were also given by body-weight changes (Fig. 2). All control groups (A–C) exhibited weight gain over the 59-day period. Weight gain was the highest in group C, in which the animals underwent the least number of operations. Group B showed less weight gain than group A, probably due to two closely spaced operations on days 10 and 17. The cisplatin-treated group lost weight initially, as expected with this cytotoxic drug.

The growth delay for rats with intraperitoneal tumours treated intraperitoneally with 4 mg/kg cisplatin amounted to 37 ± 1 days (curves shown in Fig. 1). For comparison, in a separate group of rats bearing subcutaneous CC531 tumours treated intraperitoneally with 5 mg/kg cisplatin, the growth delay was 7.1 ± 2.4 days. Subcutaneous tumours also grew faster than intraperitoneal tumours (0.54 mm/day vs 0.38 mm/day, equivalent to volume-doubling times at 6–7.5 mm of 2.7 and 3.9 days, respectively). To correct the growth delays for the difference in growth rates, "specific growth delays" were calculated, i.e. growth delay/doubling time. These were 9.7 for intraperitoneal tumours and 2.6 for subcutaneous tumours. It is therefore apparent that the intraperitoneal tumours were considerably more sensitive to cisplatin than were the subcutaneous tumours, despite their slower growth.

Cell kinetic parameters

The thymidine analog IUdR was used to monitor cell kinetics in this tumour model. Two parameter plots of IUdR content versus DNA content of tumours excised at different times after IUdR injection are shown in Fig. 3. Two populations are evident, one being diploid and the other, almost tetraploid (DNA index, approximately 1.9). IUdR incorporation (green fluorescence) was seen in both populations. Movement of the labelled cells through the cycle, i.e. towards a higher DNA content, was apparent with increasing sampling times. Unfortunately, the overlap of the diploid and aneuploid populations prevented separate derivation of accurate kinetic estimates from each population. The labelling index (LI; percentage of IUdR-labelled cells) was estimated to average 10.1% for the diploid population and 30.9% for the aneuploid population. These values are not absolutely correct due to the difficulty of assigning cells near the G1 aneuploid region to the correct subpopulation. It is clear, however, that the diploid LI was the lower, consistent with this being a non-malignant population within the tumour. This is also in agreement with flow cytometry data on in vitro cultured CC531 cells, in which only a single aneuploid population is seen (data not shown).

The cell-cycle phases could not be accurately determined. The labelling pattern changes, however, suggested that the G2M phase was <3 h (labelled cells appearing in G1 by this time) and that the DNA synthesis time was slightly longer than 7 h (the undivided labelled population would have almost reached G2 by this time; see [2]). A potential doubling time of approximately 26 h can be calculated for the presumed tumour population (aneuploid), assuming that Ts = 8 h, LI = 30.9%, and the age-distribution factor reaches unity, as compared with the actual volume-doubling time of 3.9 days (from Fig. 1). Even considering the uncertainties in parameter values, it is apparent that this tumour has a high cell-loss factor (approximately 72%) during peritoneal growth. Nevertheless, no metastases could be detected in the peritoneal cavity during the 63-day period, suggesting that the loss consisted mainly of non-viable material.

Discussion

The rat tumour model presented herein enables single intraperitoneal tumours to be assessed quantitatively for treatment response. The model was set up to simulate peritoneal metastases as part of a programme to investigate intraperitoneal chemotherapy for such tumours, a therapy presently being tested in ovarian cancer in the clinic [3, 9]. With this model, intraperitoneal chemotherapy can be compared with other forms of therapy, e.g. conventional intravenous chemotherapy. The concurrent measurements of tumour growth and animal weight loss in this model enables the estimation of a therapeutic index for each treatment. To our knowledge, this is the first time that precise and repeated measurements of tumour growth have been shown to be possible for experimental intraperitoneal tumours in the same animal. However, the use of growth-delay versus weight-loss measurements to provide a therapeutic index can be successful only if precise standardization of the laparotomy procedure is included.

The determination of tumour size was accomplished by normal caliper measurements, as usually applied for subcutaneous tumours. The necessity of an operation for each measurement was less restrictive than might be expected. The laparotomy technique could be quickly learned and applied at least once every 2 weeks. The kinetics of the tumour provided several measurements during the growth history of both control and treated tumours, leading to estimates of growth delay. Repeated laparotomies slightly reduced weight gain of the animals but produced no severe toxicity if sterile techniques and careful manipulation were used. Coupled with the lack of effect on tumour growth, this measuring procedure was judged to be an acceptable method for assessing the response of intraperitoneal tumours.

The cell kinetic measurements of the tumour in its intraperitoneal site were somewhat frustrated by the overlap of diploid and aneuploid subpopulations, but it was nevertheless clear that the tumours had a high cell-loss rate. This is also a common finding in human tumours [8] and is consistent with the epithelial origin of the tumour [4], leading to its relatively slow growth. The tumour was tested for its response to cisplatin since intraperitoneal chemotherapy with this drug and its analogues is under investigation in this institute. Present research is aimed at investigating tumour penetration by cisplatin and carboplatin after administration by the intraperitoneal and intravenous routes. The cisplatin sensitivity of the tumour system, as evidenced by the marked growth delay, makes it a suitable choice for these studies. The preliminary analysis, showing that intraperitoneal tumours appeared to be significantly more sensitive to cisplatin than subcutaneous tumours, despite a slower growth rate, merits further study in terms of comparative blood flow, drug uptake and drug distribution.

In summary, these studies show that the progress of single intraperitoneal tumours in rats can be monitored by repeated laparotomies, thus providing a suitable model for experimental chemotherapy studies.

Acknowledgements. We would like to thank C. J. van der Hoeven for assisting with the operations, M. Ruevekamp for the subcutaneous tumour experiments, Ingrid Hofland for the IUdR staining and flow cytometry analysis, and Thea Eggenhuizen for preparing the manuscript. For financial support we express our thanks to the Dutch Cancer Foundation (KWF, project NKI 86-5) and the German Cancer Foundation (Deutsche Krebshilfe).

References

1. Begg AC (1987) Principles and practice of the tumor growth delay assay. In: Kallman RF (ed) Rodent tumor models in experimental cancer therapy. Pergamon Press, Oxford, pp 114–121
2. Begg AC, Moonen L, Hofland I, Dessing M, Bartelink H (1988) Human tumour cell kinetics using a monoclonal antibody against iododeoxyuridine: intratumoral sampling variations. Radiother Oncol 11: 337–347
3. Brenner DE (1986) Intraperitoneal chemotherapy: a review. J Clin Oncol 4: 1135–1147
4. Denekamp J (1970) The cell proliferation kinetics of animal tumors. Cancer Res 30: 393–400
5. Los G, Ruevekamp M, Bosnie N, De Graaf PW, McVie JG (1990) Intraperitoneal tumour growth and chemotherapy in a rat model. Eur J Cancer Clin Oncol 25: 1057–1066
6. Hamilton TC, Young RC, Louie KG, Behrense BC, McKoy WM, Grotzinger KR, Ozols RF (1984) Characteristics of a xenograft model of human ovarian carcinoma which produces ascites and intra-abdominal carcinomatosis in mice. Cancer Res 44: 3286–3289
7. Marquet RL, Westbrook DL, Jeekel J (1984) Interferon treatment of a transplantable rat colon adenocarcinoma: importance of tumor site. Int J Cancer 33: 689–692
8. Steel GG (1977) Growth kinetics of tumours. Oxford University Press, Oxford
9. Ten Bokkel Huinink WW, Dubbelman R, Aartsen A, Franklin H, McVie JG (1985) Experimental and clinical results with intraperitoneal cisplatin. Semin Oncol 12 [Suppl 4]: 43–46
10. Thomlinson RH, Craddock EA (1967) The gross response of an experimental tumour to single doses of X-rays. Br J Cancer 21: 108–123